Persistent infection of Chlamydia in reactive arthritis

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Unravelling the molecular mechanisms

A number of bacteria have been implicated as causing reactive arthritis. In epidemiological studies Chlamydia have been identified as the most common bacteria triggering reactive arthritis in Western countries. Only 1–3% of patients acquiring infection at the urogenital tract as the primary site of infection develop Chlamydia-induced arthritis.

It has been shown that C trachomatis reaches the joint from the urogenital system through circulating monocytes and that monocytes/macrophages are the common host cells for persistent organisms during long term infection, with a major role in the induction of inflammation (fig 1). Most patients will achieve clinical remission within 6 months after infection. However, a chronic disease course occurs with intermittent relapses and periods of remission despite the presence of persistent bacteria in the joint. To date, there is no explanation for this clinical heterogeneity, but it is probably related to the genetic background of the host as well as to bacterial factors, such as the ability of C trachomatis to modify its life cycle.

CURRENT UNDERSTANDING OF THE AETIOPATHOGENESIS OF REACTIVE ARTHRITIS

During the past decade, significant progress has been made in understanding the aetiopathogenesis of reactive arthritis. Convincing evidence suggests that bacteria and microbial antigens triggering reactive arthritis persist in the joints and other reservoirs such as the entry site of the infection. Owing to intensive immunological and immunogenetic research into reactive arthritis over past decades, the present view of the disease mechanisms is mainly based on the potential role of the immune response. A cytokine imbalance and the interaction between bacteria and HLA-B27 are thought to have a major role in the failure to eliminate the triggering bacteria and the microbial antigen, leading to the disease manifestations and chronicity. In contrast, the microbiological and molecular aetiopathogenesis of the persistence of the bacteria is less well understood, although some insights into the persistent state of Chlamydia have been obtained over recent years.

In the case of C trachomatis, it has been shown that viable metabolically active organisms exist for extended periods in the joints of patients with reactive arthritis. Chlamydia has a special life cycle determined by an infectious stage (elementary bodies) outside the cells and a non-infectious stage (reticular bodies) inside the cells. Most intriguingly, the intracellular viable non-replicating Chlamydia found within monocytes isolated from the synovial fluid and the synovial membrane of patients with C trachomatis-induced arthritis showed an aberrant morphology, typical of neither the reticulate nor the elementary bodies.

‘Initial hopes of eradicating Chlamydia by antibiotic treatment have been dashed’

When Chlamydia were first identified in joints there were great hopes that antibiotic treatment would eradicate the bacteria and cure the disease, but clinical studies performed so far have been equivocal or disappointing. Recently, however, positive results have been reported from a combination of antibiotics (doxycycline plus rifampicin) and a combination of synovectomy with antibiotics (doxycycline plus rifampicin) and a combination of synovectomy with antibiotics (doxycycline plus rifampicin).

WHAT MAKES RESEARCH ON MOLECULAR MECHANISMS OF CHLAMYDIAL PERSISTENCE SO DIFFICULT?

Research on the molecular biology of Chlamydia is hampered by its obligate intracellular life form, its biphasic developmental cycle, the lack of genetic transformation systems, and the lack of suitable animal models for reactive arthritis. Therefore, only recently has an insight into the chlamydial genome become available. The genome of the C trachomatis serovar D was first published in 1998, identifying 894 likely protein coding genes among the 1 042 519 base pairs. More recently, an analysis of the chlamydial proteome has led to the identification of 328 proteins. However, the function of the majority of genes and proteins has not been elucidated so far.

Experimental approaches have involved gene expression studies comparing productive and persistent infection. One major finding was the down regulation of an outer membrane protein (omp1) in persistent infection, probably accounting for the aberrant morphology of persistent Chlamydia found in the arthritic joint. Another finding was the up regulation of the heat shock protein (hsp) 60 gene encoding for a highly immunogenic protein and thus contributing to the inflammatory response mounted against persistent Chlamydia.

Molecular studies on other bacteria have shown that persistence is a multifactorial, orchestrated process reflected by corresponding changes of the gene or protein expression pattern of the infected host cells. The screening of Chlamydia infected cells by microarrays and the detailed analyses by real time reverse transcriptase-polymerase chain reaction (RT-PCR) or enzyme linked immunosorbent assay (ELISA) showed that innate immunity and other pathological mechanisms have a role in this interaction.

For monocytes, the activation of C trachomatis seems to involve chlamydial lipopolysaccharide and Toll-like receptors (TLRs) as the corresponding sensors, on the one hand, and chlamydial effector proteins (type III secretion system) and other ‘active’ bacterial mechanisms, on the other. Within minutes after cell contact, TLR mediated host cell responses such as the release of proinflammatory cytokines, albeit not as strong as those induced by lipopolysaccharide of other Gram negative bacteria, can be seen. During the following days, other mechanisms seem to become prominent. These TLR independent interactions can best be observed in epithelial cells.
15 minutes after contact with *C. trachomatis*, tyrosine phosphorylation of host cell proteins occurs. However, drastic changes in the expression pattern of the infected host cell start after several hours, reaching a maximum after ~24 hours. Thus, *Chlamydia* induce a plethora of transcription factors, signal transduction molecules, apoptosis related genes, adhesion molecules, and cytokines in the infected cells. In epithelial cells, chlamydial persistence can be induced within several days by various modes, such as by the use of interferon gamma, penicillin G, or deprivation of essential nutrients, leading to different gene expression patterns. In the interferon gamma model, responses of persistently infected host cells to *Chlamydia* and other stimuli are attenuated, probably permitting the bacteria to escape immune responses (unpublished observations).

**NEW APPROACH TO ELUCIDATION OF GENE REGULATION SUPPORTING CHLAMYDIAL PERSISTENCE**

Another organism with a known full genomic sequence, which also causes persistent infection, is *Mycobacterium tuberculosis*. As opposed to *C. trachomatis*, this bacterium is available for genetic manipulation. Thus, the mechanisms underlying mycobacterial persistence have been studied more extensively. In a study by Sassetti *et al.*, 194 genes required for mycobacterial growth and persistence in vivo were identified. “One third of *C. trachomatis* genes tested were orthologous to genes related to mycobacterial persistence”

Now, a new approach is introduced in a study by Gérard *et al* as published in the current issue. The authors used the mycobacterial genome as scaffolding and compared it with chlamydial sequences in order to identify transcriptionally up regulated chlamydial genes supporting persistence. Of the 194 mycobacterial genes originally reported by Sassetti *et al.*, 67 (35%) chlamydial orthologous genes were identified by BLAST search, a computational alignment, and direct comparison of the two sequences.

Orthologues are defined as genes in different species directly evolving from the same ancestral locus. The chlamydial orthologues of mycobacterial persistence related genes fell into generally similar categories, such as genes encoding for cell envelope synthesis, synthesis of cofactors, transport translation, other cellular processes, regulatory functions, and uncategorised transcripts. To confirm in vitro that genes were up regulated not only during persistent infection but also during active infection, 16 orthologues from the various categories were selected for real time RT-PCR; they were tested during both infection states in *C. trachomatis* infected normal human monocytes. All 16 genes were found to be transcribed at 12 hours after infection, the active infection state. Twelve orthologues also showed fairly strong up regulation in persistent infection assayed 5 days after infection, consistent with the mycobacterial study, whereas four genes showed no or only minimal transcriptional regulation.

To go further and demonstrate clinical relevance, the actual in vivo transcription was verified by measuring the 16 genes in the synovial biopsy specimens of patients with arthritis who were PCR positive.
positive for C trachomatis in their synovial tissue. Likewise, the genes showing transcription in the monocytic model were also up regulated in the synovial samples. However, the four transcripts showing no up regulation in the in vitro model were up regulated at least to some extent in the synovial samples. Apart from the attempt to define the genes and the molecular mechanisms of persistent C trachomatis infection, this report provides further important information: in contrast with M tuberculosis, probably because of its much smaller genome, C trachomatis does not dispose of a set of genes solely inducing and maintaining a persistent infection state. Thus, it seems obvious that C trachomatis merely adapts and adjusts its level of gene expression in order to elicit this particular state of infection.

FUTURE DIRECTIONS

A number of questions remain:

- Given that the persistence of C trachomatis does induce reactive arthritis, what are the mechanisms that prevent the organism from being eliminated from the host and the joint?
- What is the exact role of the native and the adaptive immune system that prevents the organism from detection within the blood and the joint?
- How do Chlamydia modulate between normal and persistent growth?

Future research should not only attempt to answer these key questions but also needs to establish a more detailed picture of the genome-wide transcript pattern underlying chlamydial persistence in monocytic and other host cells, as well as the overall metabolic characteristics of persistence for the organism resulting from the particular gene expression pattern. Full transcriptome analysis of the monocytic model of chlamydial persistence and synovial tissue samples from patients who are PCR positive for C trachomatis at that site are promising steps, contributing to the unravelling of the molecular mechanisms that determine the pathogenesis of reactive arthritis.19,20

Moreover, the role of HLA-B27 and other genes in the induction, maintenance, and recurrence of Chlamydia-induced arthritis still has to be elucidated. Finally, therapeutics that directly target persistent Chlamydia are urgently needed to cure reactive arthritis and other chronic chlamydial infections.

CONCLUSIONS

Despite the importance of C trachomatis as a major causative agent of sexually transmitted diseases and as one of the most common bacteria involved in reactive arthritis, our knowledge of the molecular mechanisms used by Chlamydia to persist in the host and in the joint has increased very little in recent years. The success of C trachomatis as an arthritogenic pathogen is due, in part, to its ability to survive in macrophages and to establish long term persistent infection in both the host and the joint during clinically symptomatic and asymptomatic periods of infection.

Recent studies have shown that several chlamydial genes of known function are differentially expressed in persistent and active infection. The present study of Gerard et al shows a gene expression profile potentially determining and/or causing chlamydial persistence by comparing and extrapolating gene expression from a classically persisting organism, M tuberculosis.11 Further characterisation of the factors and molecular mechanisms underlying chlamydial persistent and the host-parasite interaction in the persistent state is needed to define the molecular basis of the aetiology of chlamydial-induced arthritis, and to help identify new therapeutic targets, allowing elimination of C trachomatis and, eventually, determination of a cure for the arthritis.


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